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PRIMER NOTE

Isolation and characterization of microsatellites in *Drosophila montana* and their cross-species amplification in *D. virilis*

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Abstract

We report the isolation of 20 microsatellite loci from *Drosophila montana* and their cross amplification in the relative *D. virilis*. All microsatellite loci were polymorphic in the focal species *D. montana*, with gene diversities ranging from 0.23 to 0.93. In *D. virilis* only eight loci (40%) amplified and two loci were polymorphic (10%). These markers represent the first report of microsatellites isolated in *D. montana*. They could be applied for studying population structure and phylogeography. The largest benefit, however, will be their use in studies of quantitative trait loci, such as the mapping of behavioural quantitative trait loci.

Keywords: cross-species amplification, *Drosophila montana*, microsatellite, primers

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Drosophila montana together with *D. lacicola*, *D. borealis* and *D. flavomontana* represents the montana phylad in the *D. virilis* group. The other three phylads present in the group are: the virilis phylad (including *D. virilis*, *D. americana americana*, *D. americana texana*, *D. novamexicana* and *D. lummei*), the littoralis phylad (including *D. littoralis* and *D. ezoana*) and the kanekoi phylad (represented by *D. kanekoi* only) (Spicer & Bell 2002). The species forming the virilis group are closely related and divergence time between the virilis and montana phylads has been estimated to be ~9.0 ± 0.7 million years ago (Nurminsky *et al*. 1996). *Drosophila montana* occurs in the western United States, in northern European countries and in Japan and ranges from 1400 to > 3000 m elevation (Throckmorton 1982). This species distribution makes *D. montana* an interesting target for phylogeographical and population structure studies. The virilis group has been extensively studied, mainly to assess the phylogenetic relationships within and between species (Hilton & Hey 1997; Spicer & Bell 2002). Recently, microsatellite markers were used to clarify the virilis group phylogeny and to map song quantitative trait loci (Huttunen *et al*. 2003). Very little is known about the population structure and the phylogeography of this species.

Microsatellite isolation followed standard protocols (Schlötterer 1998). In brief, genomic DNA was extracted from the *D. montana* strains 1021.18 and 1209 (collected in Nevada, USA and Oulanka, Finland, respectively) using a proteinase K digestion followed by a phenol–chloroform purification. Genomic DNA was partially digested using *Mbo*I and was size fractionated on a 1.5% agarose gel. Fragments sized between 500 and 900 base pairs (bp) were isolated by electro-elution, ligated with *Bam*HI-precut and dephosphorylated M13m18 vector and transformed in XL1-blue (Stratagene) chemically competent cells. Plaque lifting and hybridization procedures followed the method described in Schlötterer 1998. The hybridization was carried out in Church buffer (0.5 M sodium phosphate, pH 7; 7% sodium dodecyl sulphate) at 37 °C with the following γ^{32}P end-labelled di- and trinucleotides: $(GT)_7$, $(AG)_7$, $(TCA)_5$, $(GTT)_5$, $(ATT)_5$, $(TAC)_5$ and $(TCT)_5$.

Positive M13 clones including a microsatellite were sequenced using Big Dye Terminator sequencing chemistry (Applied Biosystems) on a MegaBace automatic sequencer. Primers were designed in the flanking regions of the microsatellite loci using PRIMER3_www.cgi v 0.2 (Rozen & Skaletsky 2000) to amplify the polymerase chain reaction (PCR) products of 100–180 bp. PCR products were separated on a 7% denaturing polyacrylamide gel (32% formamide, 5.6 M urea) at 2.5 W/cm and visualized by autoradiography after 12–24 h exposure. Allele sizes were determined by running a PCR slippage ladder and a known size standard adjacent to the samples (Schlötterer & Zangerl 1999).

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Table 1 Microsatellite loci identified in *Drosophila montana* and their diversity estimated in terms of expected heterozygosity (H_E) and variance in repeats number (V); the cross amplification success in *D. virilis* is also reported

Locus	GenBank accessio no.	Size range (bp)	A	T_a (°C)	Primers (5′–3′)	Repeat motif in clone	H_E	V	*D. virilis* size range (bp)
Mon1	AY521571	166–176	6	59.3	F: ACACGACAGCAAAGAGAGCA R: TTTTGTCAAATGGTGCGTG	$(AG)_{11}$	0.79	1.48	—
Mon2	AY521572	136–166	8	50.5	F: TTCCAGCCTTGCAAATTAGG R: GCTGGCCAAAAAGAGATTCA	$(TC)_{13}$	0.78	17.87	126
Mon3	AY521573	94–114	8	59	F: TTCCAGCCTTGCAAATTAGG R: TGCTGGCCAAAAAGAGATTC	$(TG)_7$	0.82	4.32	—
Mon5	AY521574	94–128	14	59	F: TTTCTCTCGAGCGGAATTTG R: TTTTCCTGCTTGTTTGCCTT	$(CAA)_7$	0.81	7.38	—
Mon6	AY521575	156–168	6	59	F: GTCCGAACCACGCAATAACT R: GCTGTTGATGATGATGAGGC	$(GTT)_7$	0.52	0.43	150–156
Mon7	AY521576	148–172	10	59	F: TCGTATTCGTGTCTTGGCTG R: AGGAGCGTCTTCTTCTTCCC	$(GA)_{13}$	0.78	16.24	—
Mon9	AY521577	164–176	6	50.5	F: GGTTACAAGTTTTGGTTGGCA R: CCCAAAGTTGGAAATGGAAA	$(TA)_8$	0.70	2.08	—
Mon10	AY521578	150–154	3	59.3	F: CTCTCTAGCACCCCTCCGAC R: TCCACACTCGACAGCTGATT	$(CT)_8$	0.62	0.47	—
Mon12	AY521579	138–140	2	62	F: GCAGACGCACAGAGTCACAG R: GTTCCGGTTTAAGGTCCTCC	$(GA)_9$	0.23	0.11	138
Mon14	AY521580	164–188	6	59.3	F: GAAGGAATGGACGAAATGGA R: GGCTCTTGGATGCTGGTAAG	$(AG)_8$	0.62	22.50	—
Mon17a	AY521581	134–162	11	56	F: ATATCTGTGCAGAGGCAGGG R: TGAAATTCAAGTGCAGCGAC	$(GT)_{10}$	0.83	4.18	—
Mon20	AY521582	144–148	4	58.5	F: GCAGCAGCCACAATATCAAA R: GGCTGCTGTTGTTAAAGGCT	$(TG)_7$	0.60	0.36	162
Mon21	AY521583	146–158	4	52	F: CGTCGCATTAGGGTCAAGAT R: AAGTGCCCAAAACCAAACAC	$(TA)_7$	0.26	1.192137	—
Mon23	AY521584	136–172	12	51	F: CGAATGCCACAAATCAACAG R: GGCTCTTTGCAGAGTTGCTTT	$(GT)_8$	0.89	13.27	140
Mon25	AY521585	128–172	12	58.5	F: GCGAAATTCGTTGGCTTAAA R: CAAATGTTGTCCGAAGACCC	$(TG)_{14}$	0.89	14.76	—
Mon26	AY521586	114–144	5	52	F: GAGTGGCAGACACAACCTCA R: GCCAACAGTGCACGTAATTTTT	$(TG)_{10}$	0.62	5.45	154
Mon29	AY521587	108–158	17	50	F: GCCTCGTCGAGCATTTTAGA R: ATGGAGTTCTTCATGCCACA	$(GT)_7$	0.93	58.36	120
Mon30a	AY521588	127–151	9	52	F: CTGTGTGTGCCTCACTTGCT R: GTTCATTTTCATAAGCGGCG	$(GT)_{15}$	0.80	7.04	—
Mon30b	AY521589	108–130	9	52	F: GACTAAGACCCTTGGCCCAT R: TGGCCATGCTCTTAATGTCA	$(GT)_{14}$	0.82	6.08	—
Mon31	AY521590	150–170	10	51	F: TTTGTCAATGTGTGCGTGTG R: GATATACACCTTGGAGCGGG	$(TG)_{16}$	0.87	2.56	151–159

A, number of alleles; T_a, annealing temperature; H_E, expected heterozygosity; V, variance in repeat number; F, forward primer; R, reverse primer; —, no amplification.

We screened 3×10^3 *D. montana* clones from genomic DNA carrying an insert. Thirty-two positive clones containing microsatellite sequences were isolated. Of these, 3% were monomorphic and 34% did not amplify properly in the target species. After removing those uninformative loci, a total of 20 polymorphic microsatellites remained (Table 1). Of the 20 polymorphic microsatellites 11 were pure and nine were interrupted motifs. Most of the clones carried a dinucleotide repeat and $(TG)_n$ was the most common repeat motif. In addition two trinucleotide repeats (seven repeats in length) were isolated. The length of the cloned microsatellites ranged between seven and 16 repeats, with a mean length of 9.95 repeats. This number is lower than for *D. virilis* microsatellites, which carried on average

12.7 repeats (Schlötterer & Harr 2000a). The microsatellite polymorphism was tested on 43 *D. montana* strains sampled covering the species distribution range (Finland, USA and Japan). The cross-species amplification success in *D. virilis* was tested amplifying nine strains sampled in China in 2001/02.

PCR was performed using γ^{32}P-labelled forward primer in 10 μL volume with 50–100 ng genomic DNA, 1 μM of each primer, 200 μM dNTPs, 1.5 mM $MgCl_2$ and 1 unit *Taq* DNA polymerase. The PCR profile was 3 min at 94 °C, followed by 30 cycles of 50 s at 94 °C, 50 s at 50–62 °C (depending on the locus — see Table 1) and 50 s at 72 °C. A final cycle of 45 min at 72 °C was performed for a quantitative terminal transferase reaction.

Some preliminary analyses were performed to estimate microsatellite diversity with MICROSATELLITE-ANALYSER (MSA) 3.12 (Dieringer & Schlötterer 2003).

Gene diversities in *D. montana* ranged from 0.23 to 0.93 and the variance in repeat number ranged from 0.11 to 58.36. This high range of variance in repeats number is mainly the result of the *Mon29* locus. If this locus is excluded from the analysis the variance in repeat number ranges between 0.11 and 22.50. The cross-amplification success observed for these markers is markedly different from a previous study, which reported the cross-species amplification of *D. virilis* microsatellites (Huttunen & Schlötterer 2002). While 80% of the loci isolated in *D. virilis* amplified in *D. montana* (50% were polymorphic) only 40% of the *D. montana* loci amplified in *D. virilis* (only 10% were polymorphic). This difference is statistically significant ($P = 0.01$, Fisher's exact test). Nevertheless, a larger number of microsatellites is required to evaluate the biological significance of this observation.

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